

NOTES

NECTAR-SUGAR COMPOSITION IN AN INDIVIDUAL OF *Ruellia peninsularis* (ACANTHACEAE).—The pioneering nectar analyses of Wykes (New Phytol. 51:210–215, 1952) and Percival (New Phytol. 60:235–281, 1961) showed that floral nectars apparently exhibit a high degree of constancy in regard to nectar-sugar composition. Percival analyzed nectars of 899 species and established that less than 7% (61 species) varied significantly from sample to sample. More recently, Walker et al. (Crop Sci. 14:235–238, 1974) and Loper et al. (HortSci. 11:416–417, 1976) have described changes in nectar-sugar ratios in *Medicago sativa* L. and *Citrus* species (*C. macrophylla*, *C. depressa*, and the hybrid Fairchild tangerine), respectively. Frey-Wyssling et al. (Experientia 10:490–492, 1954) stated that enzymes, including invertase, are secreted by nectaries and can cause changes in the sucrose:hexose ratios in nectar. It is not known presently, however, if invertase in the nectar is universally, or even commonly, responsible for such changes when they do occur. During a study of intra-plant variability of nectar composition of individual flowers on single plants, I discovered that an individual of *Ruellia peninsularis* (Rose) I. M. Johnston. (purchased at Boyce Thompson Southwestern Arboretum, Superior, Arizona), grown in a greenhouse at UTEP, showed remarkable interfloral sugar-ratio variability. Sucrose composition ranged from 27% to 79% in an initial sample from 20 flowers. The present study was done to determine if flower temperature at the time of secretion, flower age, or enzymatic activity was responsible for the differences.

Ruellia peninsularis is found near the southern tip of Baja California and in west-central Sonora, Mexico (Wiggins, Flora of Baja California, 1980). Flower size and shape are typical of the genus, and the color is purplish-blue (Exotica Horticultural Color Guide). Flowers typically open about 0300 h and the corollas are often open but sometimes not fully expanded by 0800 h. The anthers have dehisced by the time the corolla opens. Flowers usually remain fresh for about 24 h under greenhouse conditions and the corolla is shed about 1200 h the second day. Pollinators are not reported in the literature, but floral size, morphology, and color suggest large bees as the primary pollinators for this species. In an urban setting in Phoenix, Arizona, however, a plant was visited by small bees (T. F. Daniel pers. comm.).

Methods. Sugar composition of the nectar samples was determined by High-Performance Liquid Chromatography (HPLC) as outlined by Freeman et al. (Bot. Gaz. 145:132–135, 1984). All nectar samples were dried on filter paper disks for storage until analyzed. To assess the effect of flower age on nectar-sugar composition, open flowers were removed at dusk of the day preceding nectar sampling. Flowers that had opened during the night were marked the next morning and nectar-sugar compositions were followed throughout the flower duration. Eighteen flowers were sampled non-destructively at specific time intervals from November to January 1984–85. Ten flowers were sampled at 0830–0900 h, 1230–1300 h, and 1530–1600 h of the first day and at 0830–0900 h the second day. To determine whether the removal of the nectar pool at mid-day affected the sugar composition of the late afternoon sample, eight flowers were sampled at the same time intervals except that the mid-day sampling was omitted. The plant was placed in a growth chamber overnight at either 10°C, 19°C, or 30°C before the nectar samples were collected to determine if temperature at the time of nectar secretion influenced sugar composition. Sampling occurred at previously specified times during the first day of flower life.

Two experiments were conducted to determine the presence of invertase in the nectar. In the first experiment, nectar samples were collected in early morning and a portion of the sample analyzed. The remaining nectar was placed in sealed micropipets at room temperature for 19 h and then reanalyzed. In the second experiment, a 10 μ l sample of nectar was added to 90 μ l of a 20% sucrose solution as measured by a pocket refractometer. The mixture was held at 30°C. Samples were then drawn for

TABLE 1. CARBOHYDRATE COMPOSITIONS FROM NECTARS OF FLOWERS OF A SPECIMEN OF *Ruellia peninsularis*. Data are mean \pm standard deviation; ranges are in parentheses. D1 = day one; D2 = day two of sampling. F = fructose, G = glucose, and S = sucrose.

D1 0830-0900 h	D1 1230-1300 h	D1 1530-1600 h	D2 0830-0900 h
Mean % F			
14.2 \pm 2.4 (10.5-20.5)	18.5 \pm 2.6 (14.6-22.3)	21.1 \pm 4.6 (17.7-26.7)	32.1 \pm 4.2 (26.0-42.2)
Mean % G			
8.5 \pm 2.1 (4.0-12.8)	13.4 \pm 2.9 (8.1-16.5)	16.7 \pm 3.2 (12.0-23.5)	22.2 \pm 3.4 (18.0-27.5)
Mean % S			
77.3 \pm 4.1 (70.5-84.9)	68.1 \pm 5.4 (62.0-77.3)	61.2 \pm 5.8 (50.2-70.3)	45.7 \pm 6.7 (26.7-53.7)

analysis at time 0, 0.5, 1, 4, and 24 h. Sugar concentrations of nectars also were measured with a pocket refractometer during day one. Statistical comparisons of sugar compositions were by 1-way analysis of variance (ANOVA) on arcsine transformed proportions.

To determine the fate of sucrose in the nectar pool, experiments were conducted using sucrose uniformly labeled with carbon-14 (ICN, Irvine, CA, USA). The specific activity was 12.3 mCi/mM and the total activity was 50 μ Ci. The solid was dissolved in 1.0 ml of water. Five μ l of the labeled sucrose solution was added to each of six flowers and the nectar pool collected after 15 min. This determined the amount of labeled sucrose that could be recovered at time 0. Three 5 μ l aliquots were measured and counted to determine the number of dps that were being added to the nectar pool at time 0. Six other flowers were injected with the labeled sucrose solution and allowed to remain undisturbed for 8 h (1630 h). The nectar was removed from the flower and the corolla tube washed with 5 μ l of water. The total volume was measured with a Hamilton microsyringe (25 μ l). This sample was mixed thoroughly and divided into two equal samples. One portion was counted to determine the amount of activity that remained in the nectar after 8 h. The other portion was separated into the constituent sugars by HPLC and the fractions were collected for counting. Sample radioactivities were quantified by scintillation spectrophotometry using Aquasol-2 (New England Nuclear Corp., Boston, MA, USA). Emissions were determined by a variable window discriminator to exclude activity from substances other than carbon-14.

Results. The relationship between flower age and nectar composition is shown in Table 1. In no case did the 10-flower group differ from the eight-flower group at equivalent sampling intervals and, thus, the two groups were combined. The percentage of sucrose declined from a mean of 77.3% in the early morning of the first day to 45.7% in the early morning of day two. Thus, the nectar composition changed from sucrose-dominant to sucrose-rich (see Baker and Baker, *In Handbook of Exper. Poll. Biol.*, p. 117-141, 1983). The range of sucrose composition closely approximated the range found in the preliminary 20-flower sample. Fructose was always present in significantly higher quantities than glucose.

Nectar was not produced at 10°C. No significant difference in sugar composition was found in first-day flowers between constant 19°C and 30°C temperatures at 1600 h [$F(1,12) = 0.649$]. In addition, a percent sucrose comparison of first-day flowers at 1600 h from the greenhouse with the constant temperature trials at the same time interval showed no significant difference [$F(2,30) = 0.173$]. Therefore, flower temperature at the time of secretion did not significantly affect nectar-sugar composition in this case.

In the invertase activity experiments, holding a high-sucrose nectar from young flowers for 19 h had no significant effect on its sugar composition [$F(1,14) = 3.90$]. Furthermore, placing nectar samples in volumes of pure sucrose solution had no detectable effect on the mixture. Thus, there is no evidence for the presence of invertase in the nectar. Apparently, nectar must change as the flower ages. The failure of the nectar in the eight flowers to show a significant difference from the 10-flower sample (sampled at mid-day) suggests that it is constantly secreted and reabsorbed by the nectaries, rather than nectar of one composition secreted as others are removed from the flower.

The radioactive sucrose experiments showed that an average of 20.6% of the sucrose was reabsorbed by the flower during the 8 h period. This corresponds to the 20.8% decline in sucrose observed during the same period in Table 1. Furthermore, 85.2% of the radioactivity after 8 h was still in the sucrose form, with 7.8% and 7.0% present as labeled fructose and glucose, respectively. It seems that these changes are of sufficient magnitude to explain the decrease in nectar sucrose during the first day. Reabsorption of radioactive sucrose has been confirmed previously by Pederson, LeFevre, and Wiebe (Science 127:758-759, 1958) who also demonstrated that carbon-14 was translocated to other parts of the plant, particularly actively growing areas. Labeled sucrose was undetectable in the nectars of other flowers because of the low levels of radioactivity used.

Discussion. The sort of nectar changes documented herein are similar in magnitude to those recorded in Loper et al. (op. cit.) and reconfirm Baker and Baker's (op. cit.) emphasis on sampling of freshly produced nectar. Also, this study shows that single nectar samples can be misleading as to the composition of a species. Variability could arise by intrinsic physiological processes or could be related to visitation by pollinators. Possibilities in the second category include regurgitation of invertase from the digestive system of the pollinator during feeding and/or micro-organismal contamination of the nectar by the pollinator (P. G. Kevan pers. comm.). In the case of *R. peninsularis*, intrinsic physiological factors clearly are implicated. If the nectar changes described herein have a selective basis, their significance in terms of pollination success is presently obscure. Perhaps, it is a mechanism by which plants can attract a wider range of pollinators. Different groups of visiting insects would find at least some flowers with acceptable nectars. These different groups of visitors, therefore, might continue to visit flowers seeking suitable nectars and transfer pollen. The increased number of potential pollinators could help assure successful seed set in that species. Caution must be used in interpreting these data, however, because only one plant under greenhouse conditions was studied.—C. EDWARD FREEMAN, Dept. of Biological Sciences, University of Texas at El Paso, El Paso 79968-0519. (Received 28 Jan 1985; revision accepted 20 Jun 1986.)

OBSERVATIONS ON THE POLLINATION OF *Dedeckera eurekaensis* (POLYGONACEAE).—The California flora has a relatively large number of phylogenetically isolated monotypic genera with highly restricted geographic distributions. *Dedeckera eurekaensis* is a recently discovered genus of this nature (Reveal and Howell, Brittonia 28:245-251, 1976). This shrub is known only from a number of disjunct localities in the Inyo, Last Chance, Panamint, and White Mountains at the northwestern fringes of the Mojave Desert (Morefield, Madroño 32:122-123, 1985). Populations range in size from only two plants to those with scores of individuals.

The reproductive biology of species with narrowly restricted distributions is of interest because of the high risk of extinction. *Dedeckera* is of further interest because